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Differential regulation of the expression of lipid metabolism-related genes with skeletal muscle type in growing chickens

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ABSTRACT

The regulatory mechanisms of carbohydrate and lipid metabolism are known to differ among skeletal muscle types in mammals. For example, glycolytic muscles prefer glucose as an energy source, whereas oxidative muscles prefer fatty acids (FA). We herein demonstrated differences in the expression of genes involved in carbohydrate and lipid metabolism in the pectoralis major (a glycolytic twitch muscle), adductor superficialis (an oxidative twitch muscle), and adductor profound (a tonic muscle) of 14-day-old chicks. Under *ad libitum* feeding conditions, the mRNA levels of muscle type phosphofructokinase-1 were markedly lower in the adductor superficialis muscle, suggesting that basal glycolytic activity is very low in this type of muscle. In contrast, high mRNA levels of lipoprotein lipase (LPL) and fatty acid translocase/cluster of differentiation 36 (FAT/CD36) in the adductor superficialis muscle suggest that FA uptake is high in this type of muscle. The mRNA levels of adipose triglyceride lipase (ATGL) and carnitine palmitoyltransferase 1b (CPT1b) were significantly higher in the adductor profound muscle than in other muscles, suggesting that basal lipolytic activity is high in this type of muscle. Furthermore, the mRNA levels of peroxisome proliferator activated receptor δ and CPT1b were significantly increased in the adductor superficialis muscle, but not in other muscles, after 24 h of fasting. Therefore, the availability of FA in the oxidative twitch muscles in growing chickens appears to be upregulated by fasting. Our results suggest that lipid metabolism-related genes are upregulated under both basal and fasting conditions in the adductor superficialis in growing chickens.

1. Introduction

Skeletal muscle is known to be a major disposal site of glucose and fatty acid (FA), in which energy substrates are shifted depending on the nutrient conditions (Abdul-Ghani and DeFronzo, 2010; Furler et al., 2000). For example, the postprandial increase of plasma glucose stimulates insulin secretion from β-cells in the pancreas, and the resultant higher plasma insulin levels promote glucose uptake into myocyte while decreasing lipid oxidation (Abdul-Ghani and DeFronzo, 2010). The glucose uptake is mediated by the glucose transporter 4 (GLUT4), which is a major GLUT isoform in mammalian skeletal muscle (Abdul-Ghani and DeFronzo, 2010; Schiaffino and Reggiani, 2011), and then glucose is metabolized by the glycolytic pathway. In this pathway, the muscle type of phosphofructokinase-1 (PFK-M) acts as a rate-limiting enzyme (Schiaffino and Reggiani, 2011). On the other hand, FA is utilized as a principal fuel source in skeletal muscle under low plasma glucose conditions. Peroxisome proliferator-activated receptor δ (PPARδ), which is in the nuclear receptor family of ligand-activated transcription factors, plays a pivotal role in the metabolic adaptation of skeletal muscle to fasting (Nakamura et al., 2014; Neels and Grimaldi, 2014). PPARδ induces the gene expression of carnitine palmitoyltransferase 1b (CPT1b), which is a rate-limiting enzyme of mitochondrial FA oxidation. In addition to mitochondria, peroxisomes are another important organelle in charge of FA oxidation, and acyl-CoA oxidase (ACO) acts as a rate-limiting enzyme in peroxisomal FA oxidation. Adipose triglyceride lipase (ATGL), which is a rate-limiting enzyme in intracellular triacylglycerol (TG) hydrolysis, is also crucially involved in intramuscular lipolysis during muscle contraction (Shaw et al., 2013).

De novo lipogenesis and FA uptake from plasma constitute an important part of lipid metabolism in skeletal muscles. For example, glucose was previously shown to be converted to non-esterified FA (NEFA), diacyglycerol, and TG under normal glucose concentrations (5.5 mmol/L) in human skeletal muscle cells *in vitro* (Aas et al., 2004). Sterol regulatory element binding protein 1 (SREBP1), a membrane-bound transcriptional factor, has been shown to induce a line of lipogenic

Abbreviations: ACO, Acyl-CoA oxidase; ATGL, Adipose triglyceride lipase; CPT1, Carnitine palmitoyltransferase 1; FAS, Fatty acid synthase; FAT/CD36, Fatty acid translocase/cluster of differentiation 36; GLUT, Glucose transporter; LPL, Lipoprotein lipase; PFK, Phosphofructokinase; PPAR, Peroxisome proliferator activated receptor; RPS17, Ribosomal protein S17; SREBP1, Sterol regulatory element binding protein 1.

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genes including fatty acid synthase (FAS) in skeletal muscles as well as in the liver and adipose tissue. Lipoprotein lipase (LPL) hydrolyzes plasma TG, with the resulting NEFA being taken up into skeletal muscle by fatty acid translocase/cluster of differentiation 36 (FAT/CD36) as well as into adipose tissue (Nahlé et al., 2008; Nickerson et al., 2009). Therefore, the expression of these genes in skeletal muscles is involved in metabolic adaptation to nutritional changes.

In mammals, skeletal muscles are composed of four fiber types classified by physiological and biochemical properties; oxidative slow-twitch type I, oxidative fast-twitch IIA, and glycolytic fast-twitch IIB and IIX/D. Oxidative muscle fibers show a higher FA oxidative and uptake rate than glycolytic fibers due to higher mitochondrial density and FAT/CD36 expression at mRNA and protein levels (Schiaffino and Reggiani, 2011). For example, the enzyme activity of CPT1 and palmitate transport rate were previously shown to be higher in oxidative muscles than in glycolytic muscles (Kim et al., 2002; Chabowski et al., 2006). In contrast, previous studies demonstrated that glycolytic genes including PFK-M were highly expressed in glycolytic muscle fibers (Chemello et al., 2011; Schiaffino and Reggiani, 2011). Furthermore, PFK enzyme activity was shown to be higher in the glycolytic fibers than that in the oxidative fibers (Schiaffino and Reggiani, 2011).

In chickens, fiber types have been classified as types I, IIA, IIB, IIIA, and IIIB by enzyme histochemical methods (Barnard et al., 1982). Similar to mammals, types I and IIA are oxidative twitch fibers, and type IIB is a glycolytic twitch fiber in chickens. Type III fibers, which are rare in mammals, are tonic fibers that are expressed in the anterior latissimus dorsi, adductor profound, and plantaris muscle in chickens (Barnard et al., 1982). However, it currently remains unclear whether type III fibers are oxidative or glycolytic fibers (Barnard et al., 1982; Remignon et al., 1994). In addition, the expression of several genes involved in carbohydrate and lipid metabolism has been shown to differ between chicken and mammalian skeletal muscle. For example, GLUT1, GLUT3, and GLUT8 are expressed in chicken skeletal muscle, whereas the GLUT4 gene is absent in chicken genome (Kono et al., 2005; Seki et al., 2003). GLUT1 mRNA levels, but not those of GLUT3 and GLUT8, were previously reported to be decreased in the pectoralis major muscle by restricted feeding (Zhao et al., 2012). PFK-M and PFK-L (liver type) are expressed in the skeletal muscle of chickens (Seki et al., 2006). Skiba-Cassy et al. (2007) also reported the absence of fasting stimulation of CPT1b mRNA expression in the pectoralis major muscle. Therefore, species-specific and/or muscle fiber type-dependent regulation of the expression of genes related to carbohydrate and lipid metabolism may occur in the skeletal muscles of chickens.

In the present study, we compared the mRNA levels of carbohydrate and lipid metabolism-related genes in different types of muscles (pectoralis major, adductor superficialis, and adductor profound muscles) in growing chickens under feeding and fasting conditions.

2. Materials and methods

2.1. Animals and feeding

Day-old male chickens (Ross308) were purchased from local hatcheries (Ishii Poultry Farming Cooperative Association, Tokushima, Japan). They were given free access to water and a commercial chicken starter diet (23.5% crude protein and 3,050 kcal/kg; Nippon Formula Feed Mfg. Co. Ltd., Kanagawa, Japan) until the experiment was conducted. This study was approved by the Institutional Animal Care and Use Committee and carried out according to the Kobe University Animal Experimental Regulation.

2.2. Sampling and preparation

Sixteen 13-day-old chickens were weighed and allocated based on body weight to two groups of 8 birds. Each group had an average body weight of 313.3 ± 5.8 g. On the next day, one group was maintained as *ad libitum* feeding condition and the other group was deprived of food for 24 h. Then, body weights were measured, and chickens were sacrificed by decapitation. Blood was collected from the carotid artery. Plasma was separated immediately by centrifugation at 3,000 *g* for 10 min at 4 °C, and the plasma concentrations of glucose, NEFA, TG, and insulin were measured using a commercial kit (Labassay™ Glucose, Labassay™ NEFA, and Labassay™ Triglyceride; Wako Pure Chemical Industries, Ltd., Osaka, Japan; Rat Insulin ELISA KIT (TMB), Shibayagi, Gunma, Japan). The pectoralis major, adductor superficialis, and adductor profound, which consist almost completely of type IIB fibers (>99%), mainly of type IIA fibers (80%–95%), and completely of type III fibers (100%), respectively (Barnard et al., 1982), were excised. The dissected muscles were immediately frozen with liquid nitrogen and stored at −80 °C for real-time PCR analysis.

2.3. Real-time PCR analysis

Total RNA was extracted from the muscles using Sepazol-RNA I (Nacalai Tesque, Inc., Kyoto, Japan). First-strand cDNA was synthesized from total RNA using ReverTra Ace® qPCR RT Master Mix with gDNA Remover (Toyobo Co. Ltd., Osaka, Japan). mRNA levels were analyzed by relative standard curve method using Thermal Cycler Dice® Real Time System (Takara Bio Inc., Otsu, Japan), each primer (Table 1), and THUNDERBIRD® SYBR® qPCR Mix (Toyobo Co. Ltd., Osaka, Japan) according to the supplier's recommendations; 95 °C for 1 min, 40 cycles of 95 °C for 10 s and 60 °C for 1 min. The expression levels of target genes were normalized to those of ribosomal protein S17 (RPS17) as in a previous study (Honda et al., 2010).

2.4. Statistical analysis

Data on gene expression levels under feeding conditions were analyzed using the Tukey–Kramer method. The Student's *t*-test was performed to analyze differences in body weight, plasma components, and mRNA levels between feeding and fasting conditions. All statistical analyses were performed using Excel 2013 (Microsoft, USA) with the add-in software Statcel 3 (OMS, Tokyo, Japan).

3. Results

Changes in body weight and plasma concentrations of glucose, NEFA, TG, and insulin after 24 h of fasting are shown in Table 2. Body weight and plasma concentrations of glucose, TG, and insulin were significantly ($P < 0.05$) decreased by fasting. In contrast, plasma NEFA concentrations were significantly ($P < 0.05$) increased after fasting.

The results obtained for the mRNA levels of carbohydrate metabolism-related genes are shown in Fig. 1A, B, and C. PFK-M mRNA levels were significantly ($P < 0.05$) higher in the pectoralis major muscle than in other muscles under feeding conditions. After 24 h of fasting, the mRNA levels of PFK-M and GLUT1 were significantly ($P < 0.05$) increased in the adductor superficialis muscle only. In contrast, GLUT1 expression tended to be decreased by fasting in the pectoralis major muscle ($P = 0.07$). No significant difference was observed in the mRNA levels of PFK-L.

Fig. 1D–K show the results for expression of lipid metabolism-related genes. The mRNA levels of ATGL, FAT/CD36, and LPL were significantly ($P < 0.05$) higher in the adductor superficialis muscle than in the pectoralis major muscle under feeding conditions. In the adductor profound muscle, the mRNA levels of ATGL, CPT1b, and FAT/CD36 were significantly ($P < 0.05$) higher than those in other muscles under feeding conditions.

After 24 h of fasting, the expression of FAT/CD36 was significantly ($P < 0.05$) increased in the adductor superficialis and profound muscle and tended to be increased in the pectoralis major muscle ($P = 0.10$), whereas that of CPT1b was significantly ($P < 0.05$) increased in the adductor superficialis only. ATGL mRNA levels were significantly